Inventories and Significance of the Genetic Resources of an African Mahogany Species (*Khaya senegalensis* (Desr.) A. Juss.) Assembled and Further Developed in Australia.

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Inventories and Significance of the Genetic Resources of an African Mahogany Species (*Khaya senegalensis* (Desr.) A. Juss.) Assembled and Further Developed in Australia

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Abstract

The forest tree species *Khaya senegalensis* (Desr.) A. Juss. occurs in a belt across 20 African countries from Senegal-Guinea to Sudan-Uganda where it is a highly important resource. However, it is listed as Vulnerable (IUCN 2015-3). Since introduction in northern Australia around 1959, the species has been planted widely, yielding high-value products. The total area of plantations of the species in Australia exceeds 15,000 ha, mostly planted in the Northern Territory since 2006, and includes substantial areas across 60-70 woodlots and industrial plantations established in north-eastern Queensland since the early-1990s and during 2005-2007 respectively. Collaborative conservation and tree improvement by governments began in the Northern Territory and Queensland in 2001 based on provenance and other trials of the 1960s–1970s. This work has developed a broad base of germplasm in clonal seed orchards, hedge gardens and trials (clone and progeny). Several of the trials were established collaboratively on private land. Since the mid-2000s, commercial growers have introduced large numbers of provenance-bulk and individual-tree seedlots to establish industrial plantations and trials, several of the latter in collaboration with the Queensland Government. Provenance bulks (>140) and families (>400) from 17 African countries are established in Australia, considered the largest genetic base of the species in a single country outside Africa. Recently the annual rate of industrial planting of the species in Australia has declined, and R&D has been suspended by governments and reduced by the private sector. However, new commercial plantings in the Northern Territory and Queensland are proposed. In domesticating a species, the strategic importance of a broad genetic base is well known. The wide range of first- and advanced-generation germplasm of the species established in northern Australia and documented in this paper provides a sound basis for further domestication and industrial plantation and woodlot expansion, when investment conditions are favourable.

Keywords: provenance, clone, progeny, seed orchards, conservation, woodlots

Introduction

The African mahogany forest tree species *Khaya senegalensis* (Desr.) A. Juss., (hereafter referred to as Ks), occurs naturally within 20 African countries in a seasonally-dry belt from Senegal-Guinea in the west to Sudan-Uganda (see Figure 1) in the east (Orwa et al. 2009, IUCN 2015-3, Sexton et al. 2015) (see Figure 1). It is rated 10th among the world's 20 most widely used and prioritised tree species suitable for development of forest industries and planted forests (FAO 2014). The species is well known in global wood markets and is an important multi-purpose resource across its range (Nikiema and Pasternak 2008, Orwa et al. 2009, Sexton et al. 2010). However, it is classified as Vulnerable by the IUCN due to loss of habitat and over-exploitation, and effective regeneration is generally poor (Sokpon and Ouinsavi 2004, Nikiema and Pasternak 2008, Fremlin 2012, Sexton 2013, IUCN 2015-3).

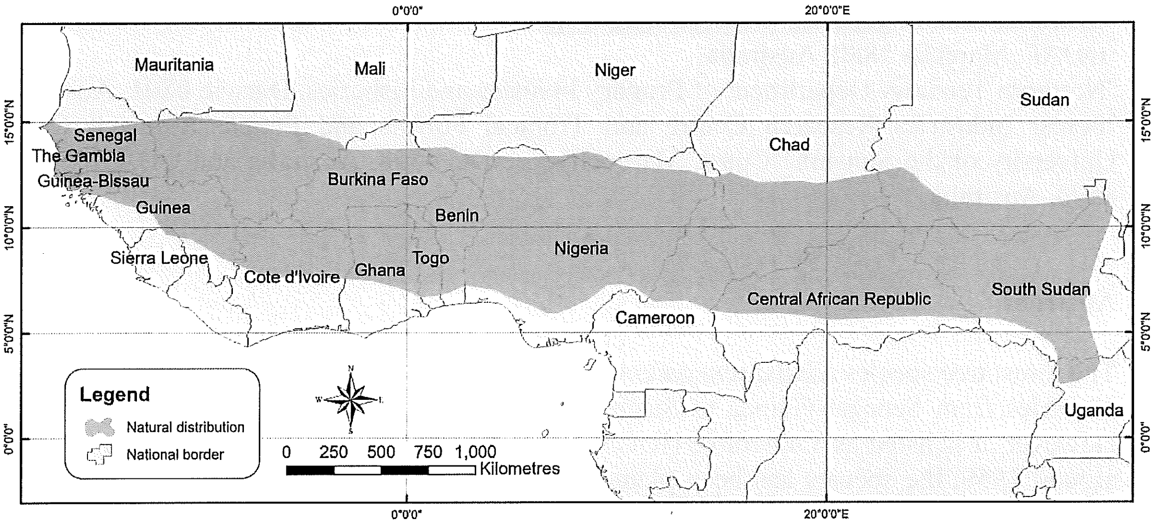

Figure 1. Approximate natural distribution of *Khaya senegalensis* within 20 countries in Africa.

In northern Australia, defined for this paper as north of latitude 20° S, vast tracts of land are climatically suitable for this species (Arnold et al. 2004, Figure 2 of Nikles et al. 2008). Since introduction to the region around 1959, Ks has been planted widely for amenity, research trials, woodlots, rehabilitation of mined areas and industrial plantations. The total area of the species planted in northern Australia exceeds 15,000 ha (Penfold 2015) with more than 13,000 ha of industrial plantations established in the Douglas Daly, Northern Territory (NT) (see Figure 2) since 2006 (Miller 2013) and 386 ha north of Cooktown in north eastern Queensland (Qld) between 2005 and 2007 (Nikles 2015a) (see Figure 2). Trials established in Western Australia (WA) in 1987-1999 and industrial plantings of 2006-2009 (ca. 100 ha) (Dumbrell et al. 2011, Dumbrell 2014) have been harvested commercially mostly as veneer logs (Fremlin 2015). More than 250 ha of woodlots across 60-70 ownerships have been established in north-eastern Queensland, mainly between Ingham and Bowen, since the early-1990s (Dickinson and Nikles 2011). A significant area of stands of the 1960s-1980s and trials of the mid-2000s remain on mined land at Weipa (Qld) (Annandale 2015a, b). Plantation trees yield high-value wood products (Armstrong et al. 2007, Nikles et al. 2008, Zbonak et al. 2010). For example, timber from 32-year-old NT plantation-grown Ks trees supplied to various industry assessors in eastern Australia in 2004 was judged to have 'good prospects on the domestic market in the future, and could conservatively be retailed for between \$3,000 and \$5,500/m^3 for dried, dressed Medium Feature to Select grade timber' (Armstrong et al. 2007, p viii; and see pp 36-40). Furniture crafted from the plantation Ks timber has won state and national awards (Nikles et al. 2008).

New hardwood plantation establishment in southern Australia has been in steep decline since 2006-2007 and by 2012-2013 the total area across hardwood and softwood resources recorded a net decrease (ABARES 2014). In contrast, industrial planting of Ks began in northern Australia in the mid-2000s and annual planting has only recently declined from 1500 ha/year to 400 ha/year (Penfold 2015). Anecdotal information indicates several thousand hectares of new commercial plantings of Ks are proposed for the NT and Qld.

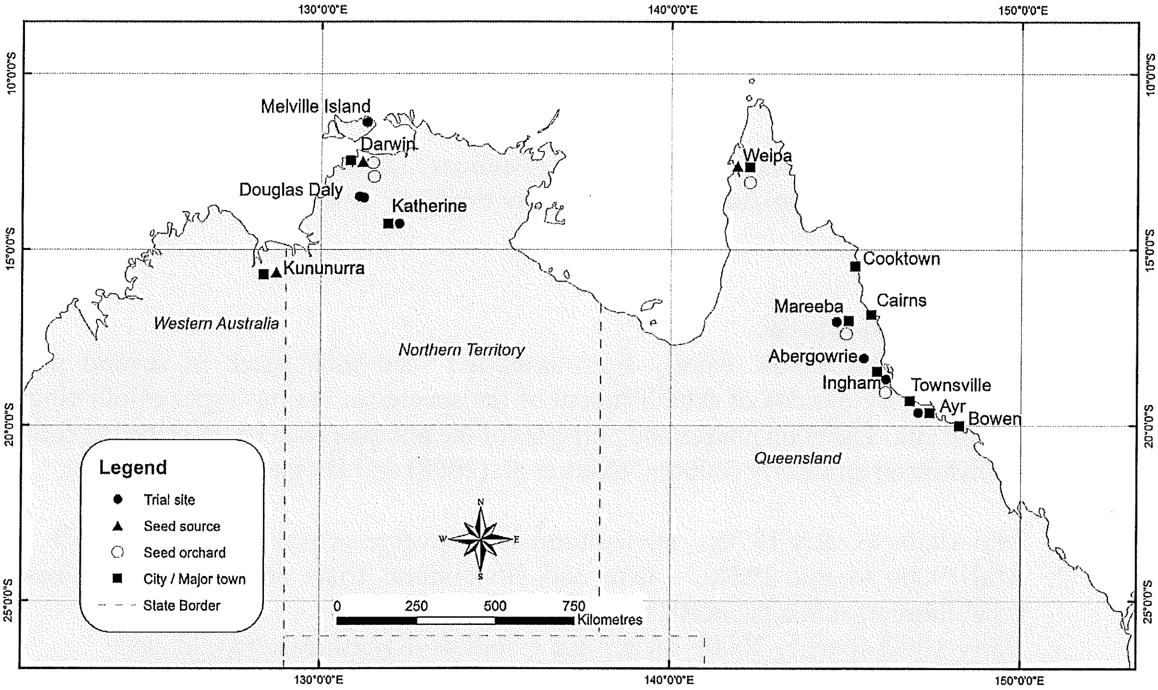

Figure 2. Locations of genetic resources plantings of *Khaya senegalensis* in northern Australia that include industrial plantations in the Douglas Daly and near Cooktown and Kununurra.

Collaborative conservation and tree improvement of Ks by Governments began in the NT and Qld in 2001 (Nikles 2006, Nikles et al. 2008). The initial base population in the NT comprised small stands planted near Darwin in the 1960s–1970s representing 21 provenances from 10 African countries, unknown African sources and three provenances from New Caledonia ex Ivory Coast. Stands of two unidentified African provenances formed the base at Weipa, Qld (Bragg et al. 2004). Trees combining good growth and/or form were selected mostly during 2001 to 2003 (143 around Darwin and 36 at Weipa). Small clonal seed orchards (CSOs) and hedge gardens were established in the NT and north-eastern Qld between 2001 and 2005 (Nikles et al. 2008); and a CSO, hedge garden and seedling seed orchard were planted at Weipa in 2004 (Bragg et al. 2004). Orchard seed and other seedlots have enabled establishment of advanced-generation progeny and clone trials since 2005, some planted collaboratively on lands of the private sector (Nikles et al. 2014). The status of Ks domestication, R&D outcomes and challenges have been documented in Workshop Proceedings (Bevege et al. 2004, 2006), Reilly et al. (2006), Nikles et al. (2008) and in Abstracts of a Forum (Dickinson et al. 2011). Additional collaborative research was undertaken in Qld and the NT on clonal technologies, efficient phenotyping and molecular diversity under the National and International Research Alliances Program, Smart Forests Alliance Queensland and detailed results have been reported (see Wallace et al. 2012). The status of domestication in 2014 was outlined by Nikles (2015b). Investment in Ks R&D by governments ceased in 2011 due to changed priorities; and it has been scaled down recently by industry (Fremlin 2015).

Since the mid-2000s, private growers have introduced large numbers of new provenances as bulk and individual-tree seedlots to establish industrial plantations and trials (Dickinson et al. 2009). This paper updates the inventory of 2009 and includes details of the clonal seed orchards, hedge gardens, and provenance, clone and progeny trials established by January 2013. It also describes the significance of the genetic resources assembled and further developed in Australia, considered to be the largest genetic base of the species in a single country outside Africa.

Genetic Resources of Ks Assembled in Australia

There are primary and advanced-generation resources. Locations of plantings of genetic resources of Ks in northern Australia and nearby towns or cities mentioned in the text are shown in Figure 2.

Primary Genetic Resources

Plantings established almost wholly from imported seed took place in several phases reflecting successive periods of establishment of the species in various types of plantings in northern Australia. The main phases and purposes of the plantings can be described as follows based on Dickinson and Kelly (2006), Nikles et al. (2008) and Dickinson et al. (2009):

1. Mid-1960s to early 1970s – silvicultural and provenance trials near Darwin, NT;
2. Mid-1960s to mid-1980s – taxa and silvicultural trials and extensive mine-site rehabilitation at Weipa, north western Cape York, Qld;
3. Early-1990s to early 2000s – trials and woodlots in north-eastern Qld; and
4. Since mid-2000s – industrial plantations in the NT, Qld and WA; provenance trials in the NT and Qld; and a family-in-provenance trial in Qld.

In phases 1-3, provenances were not identified precisely to specific geographic coordinates of locations within known country of origin, nor were the numbers of seed parents per provenance provided by the African suppliers of seed. Stands established from seed of all phases remain, though some are diminished to varying degrees by fire, land redevelopment and partial harvesting at Weipa, Qld; cyclone and land redevelopment near Ingham, Qld; and land redevelopment in the NT.

Provenances Represented in CSOs

Tree selection between 2001 and 2003 in stands from seed importation phases 1 and 2 contributed above-average trees for growth and/or form for CSO establishment as follows:

a. One hundred and thirty-six clones from trees selected in trials in the Darwin region, NT tracing to 21 known provenances from 10 African countries across the species' range plus three provenances from New Caledonia (thought to have originated in Ivory Coast);
b. Thirty-six clones from trees selected at Weipa in stands of two African provenances (Bragg et al. 2004) considered to be different from those of (a).

Six clones from trees selected in Qld woodlots likely of Burkina Faso origin were added to the program later. After Tropical Cyclone Yasi (2011, Category 5), 24 trees were selected in a families-in-provenances trial of 17 provenances from six African countries planted near Ingham, Qld in 2008 by a private grower: he established grafts of these trees in a CSO (Sexton 2013, 2015). Table 1 has details of the five CSOs developed – Berrimah, Howard Springs, Walkamin, Weipa and near Ingham - that incorporate a total of 202 unique clones.

More than one tree was selected in most provenances (Table 1), so there could be a level of co-ancestry among CSO clones from such provenances and some inbred seedlings among the progeny. Anecdotal observations of CSO progeny suggest that Ks seedlings exhibiting severe symptoms of inbreeding are rare. The first substantial collection of orchard seed was from the NT CSOs in 2008 (age 7.5 years). Families from 40 of these clones are established in progeny trials described below in the section Second-generation Genetic Resources. There is potential to establish progeny trials of a further 162 orchard clones.

Table 1. Number of provenances and grafted clones from trees selected near Darwin, Northern Territory and in north Queensland and established in the Northern Territory and Queensland Governments' collaborative clonal seed orchards by country of provenance origin, orchard, year of first planting and area[a]

Country of origin	Number of provenances	Number of clones Berrimah, near Darwin, NT 2001, 0.4 ha	Number of clones Howard Springs, near Darwin, NT 2001, 1.2 ha	Number of clones Walkamin, near Mareeba, Qld 2003, 1.3 ha
Benin	1	2	1	0
Burkina Faso	2	13	13	14
Central African Republic	1	6	6	4
Chad	1	3	3	0
Ghana	1	10	10	5
Ivory Coast	2	5	5	4
New Caledonia[b]	3	12	13	4
Nigeria	2	11	11	5
Senegal	4	31	31	15
Sudan	2	10	11	4
Togo	1	6	6	4
Uganda	4	17	17	9
Unknown	NA[c]	8	9	6
Total	24	134	136	74[d]

[a] Modified from Dickinson et al. (2012). A fourth clonal seed orchard (CSO) of 36 clones was established at Weipa, Qld in 2004 through collaboration between the Queensland Government, the Napranum Land and Sea Management Centre and the company COMALCO (Bragg et al. 2004). Identities of the two African provenances represented are unknown. [A seedling seed orchard planted at Weipa in 2004 (Bragg et al. 2004) has been lost]. A fifth CSO was established privately near Ingham in 2011-2012 with 24 clones of 17 provenances from 6 African countries (Sexton 2015)

[b] Three seedlots from New Caledonia ex Ivory Coast origin

[c] Not available. Possibly included among the known provenances

[d] Includes a 68-clone sub-set of the 136 clones of the Howard Springs orchard and six (6) clones from trees selected in Queensland. The total number of unique clones across all five orchards is 136+ 6 + 36 + 24=202.

Seed from the NT and Walkamin CSOs is expected to exhibit high quantitative and molecular genetic diversity because of the diverse parental provenances and the observation that flowering times among the clones are essentially synchronous (Dickinson et al. 2012). Hence much of the CSO seed could represent inter-provenance crosses. Supporting these expectations are high variation in progeny trials (see the section **Exhibit High Genetic Variation**), and studies of nuclear and chloroplast loci, that included samples from CSO clones, which revealed high genetic diversity, especially in material of western-region provenances (Karan et al. 2012, Sexton et al. 2015).

Provenance Bulks and Individual-tree Seedlots Introduced During Phase 4

Dickinson et al. (2009) reported that 116 unique provenance bulk seed collections were represented across the domestication program of the NT and Qld Governments and the commercial and R&D programs of Great Southern Limited. The inventory showed 15 African countries had been sampled and three seedlots obtained from New Caledonia of Ivory Coast origin. Additional provenances were introduced in the 2000s by other companies [African Mahogany Australia (Fremlin 2014) and Northern Tropical Timbers (Nikles 2007)] and by a private grower (Sexton 2013, 2014). Numbers of provenance bulks established in the field to the end of the 2012-13 planting season are documented in Table 2.

Most of the seed for these plantings was from the western region[7] of the range in sub-Saharan Africa, especially Mali, Burkina Faso and Senegal (Table 2). Almost all bulks were from 25 or more parent trees. The seed used for plant production for the industrial plantations was a mixture of known provenances that were available at the time (Fremlin 2008).

The total number of provenances established in Australian plantings, representing 17 African countries, now stands at over 142 (Table 2) as the plantings near Ingham, Qld since Oct., 2008 may have additional material (Table 3). It is assumed that introductions of the 1960s and early 1970s, for which precise provenance details are not available, were from different provenance populations from those introduced more than 30 years later, i.e. in 2001 and, mostly, after 2004. Though Guinea Bissau, Mauritania and Sierra Leone are not represented, occurrences of Ks in those countries are relatively minor and provenances from neighbouring countries are well represented in Australia. The inventory of established provenances represents a decline from the 181 introduced due to the recent loss of 39 unique provenances through redevelopment of land on which a trial of 71 African provenances was planted in the NT in 2009 (Fremlin 2014). Another loss of provenance material occurred in 2014 through redevelopment for agriculture of a trial planted in 2008 near Abergowrie, Queensland. This trial had suffered major damage in February 2011 from Tropical Cyclone Yasi (Category5). Fortunately all 38 provenances included in this trial remain represented in companion trials in the NT (Table 3).

Table 2. Approximate inventory of provenances of *Khaya senegalensis* introduced from 17 of the 20 countries where the species occurs naturally in Africa and established in Australian plantings to 2012-13.

Country of origin	NT–Qld Government program [a]	Commercial growers[a, b]	Total
Benin	1	5	6
Burkina Faso	9	21	30
Cameroon	0	9	9
Central African Republic	1	2	3
Chad	1	1	2
Gambia	0	1	1
Ghana	1	4	5
Guinea	0	4	4
Guinea Bissau	0	0	0
Ivory Coast	5 [c]	0	5
Mali	0	60	60
Mauritania	0	0	0
Niger	0	8	8
Nigeria	2	2	4
Senegal	4	16	20

[7] Defined here arbitrarily as west of longitude 0° (the approximate western edge of the Dahomey Gap, an hypothesized barrier to gene flow – Karan et al. 2012, Sexton et al. 2015). Other regions suggested here are: central - 0° to 20° E; and eastern - east of 20° E.

Sierra Leone	0	0	0
Sudan	1	1	2
South Sudan	1	10	11
Togo	1	2	3
Uganda	6	2	8
Totals	33	148 (109 net) [d]	181 (142 net) [d]

[a] Updating of Dickinson et al. 2009. Assumes introductions of the 1960s and early 1970s were from different provenance populations from those introduced more than 30 years later, i.e. since 2000

[b] Most of these provenances were introduced after 2004. The status of stands of 70 additional provenances established near Ingham beginning in Oct., 2008 is yet to be assessed (Table 3, Sexton 2015)

[c] Three seedlots of secondary origin (New Caledonia)

[d] Provenances (39) lost to land redevelopment before trees could be selected and grafted were from: Benin 4, Burkina Faso 4, Mali 20, Niger 6 and Senegal 5. Hence the net total for column 3 is 109 and the net total for column 4 is 142 – subject to revision upwards when results of the assessments of [b] are available

Individual-tree seedlots have also been introduced - from Burkina Faso (22 seedlots across several provenances), several African countries (ca. 400 seedlots across many provenances) and South Sudan (19 seedlots across two provenances) [respective records of Department of Agriculture and Fisheries (DAF), Brisbane; Sexton (2013); Fremlin (2014)]. The suites of first-generation families derived from these importations were mostly incorporated in progeny trials together with advanced-generation families. For convenience, families of all generations are documented together in Table 4. All the progeny trials include families from different provenances planted together. Since it has been observed that flowering of CSO clones of widely different provenances is essentially synchronous (Dickinson et al. 2012), it is likely that seed collected within the progeny trials would include inter-provenance crosses.

Of special interest is the trial of about 375 individual-tree families established as families-in-provenances by GJ Sexton near Ingham, Queensland, in 2008 (Sexton 2013). This trial was also struck by Tropical Cyclone Yasi and consequent flooding. However, a few families exhibited a reasonable degree of wind firmness and individuals within them that had previously shown superior growth and form were selected and established in a clonal seed orchard (see Table 1, footnote a). Felling severely damaged trees at ground level and subsequent reduction of coppice to a single leading stem resulted in successful stand re-establishment (Sexton 2013; GJ Sexton, University of Qld, 2014, pers. comm.).

Inventory of Recent Provenance Trials

Table 3 documents Ks provenance trials established in northern Australia during 2001- 2013.

Table 3. Inventory (2014) of *Khaya senegalensis* provenance trials ordered by month and year of planting in the Northern Territory (NT) and Queensland (Qld) since 2001 at approximate locations shown in Figure 2

Trial location	No. of African provenances[a]	Month, year of planting	Plant owner, Land owner	Approx. net area (ha)
Walkamin, near Mareeba, Qld	5	Mar., 2001	Public, Public	0.7
Douglas Daly, NT	9	June, 2006	Private, Private	4.9
Douglas Daly, NT	11	Mar., 2007	Private, Private	2.3
Melville, Is., NT[b]	25	Jan., 2008	Private, Private	6.4
Douglas Daly, NT	38	Jan., 2008	Private, Private	7.7
Douglas Daly, NT	38	Mar., 2008	Private, Private	8.9

Abergowrie[c], near Ingham, Qld	38	Apr., 2008	Private, Private	8.5
Near Ingham [b], Qld	70	Oct., 2008 and later	Private, Private	13.0
Douglas Daly, NT[d]	71	Feb., 2009	Private, Private	10.4
Douglas Daly, NT	12	Feb., 2011	Private, Private	5.0
Douglas Daly, NT	11	Jan., 2012	Private, Private	5.0
Douglas Daly, NT	6	Jan., 2013	Private, Private	4.9

[a] Numbers given are accounted for in Table 2 except for provenances planted near Ingham since Oct., 2008
[b] Status of trial/s to be assessed
[c] Trial lost due to land redevelopment in 2014 after severe damage from Tropical Cyclone Yasi in 2011; all provenances remain elsewhere
[d] Trial (including 39 unique provenances) lost due to land redevelopment in 2013

Almost all provenance bulks included in the trials were from 10 or more than 25 parent trees. The initial numbers of trees of each provenance planted in these replicated trials commonly ranged from 150 to 240. In such trials, substantial numbers of trees are available for screening to select superior trees for conservation and tree improvement. For example, the trial planted in the Douglas Daly, NT in January 2008 contained 8,880 trees initially, across 37 African provenances each represented by 240 trees (Dickinson et al. in prep.).

Although full pedigrees of selections made in these stands would be unknown, methods of pedigree reconstruction (Isik 2014) might be used to determine co-ancestry among selected trees and allow decisions on how to use related trees effectively in a breeding context. Meanwhile, a simple tactic to limit co-ancestry in seed orchards would be to select a restricted number of trees per known provenance.

Advanced-Generation Genetic Resources

Many plantings of trials and woodlots since the late-1980s were from seed of Australian trees, commonly from trees around Darwin, with full details of sources unrecorded. Here, most attention is given to trial plantings of seedlings, cloned progeny or clones derived from trees selected in Australia, mostly of known provenance (Tables 1, 4, 5).

Hedge Gardens

A hedge garden was initiated at the Berrimah Research Farm near Darwin (NT) in 2004 with the planting of diverse seedlings and, later, rooted cuttings from stump coppice of a few of the trees selected for CSOs of which 38 were felled in 2003 for a study of log and wood properties (Armstrong et al. 2007). Still later, more clones from stump coppice and material from other sources were added.

For the NT clone trials described below, a variable number of cuttings (commonly 2-6 per hedge) were taken from among the following 536 hedges.

a. Seedling progeny of 11 of the trees selected near Darwin for CSOs: 350 hedges
b. Bulked seedling progeny of ca. 20 of the 36 CSO trees selected at Weipa: 112 hedges
c. Bulked wildling seedlings (Controls), of an estimated 10 seed parents different from those of (a), many uplifted from within stands where most of the 136 trees had been selected for the NT CSOs; and six seedlings from a Katherine, NT nursery: 66 hedges
d. Rooted cuttings from stump coppice of three Darwin select trees (two not represented by progeny in the hedge garden) and from one rooted cuttings tree: 8 hedges.

From the above, it is estimated that hedges 1 to 536 trace to 44 parental genotypes - 41 with seedling progeny and three with ramets from stump or branch coppice. Across these hedges, seven provenances are represented from Burkina Faso, Ghana, Senegal, Uganda and Ivory Coast via New Caledonia. Subsequent additions to the Berrimah hedge garden included: a further 10 clones ex stump coppice of Darwin select trees; seedlings from 10 families of a South Sudan provenance (Kagelu); and seedlings from a locally-produced controlled cross and open-pollinated seedlings from one of the parents. None of these additional hedges, accounted for in the 630 hedges of Table 4, has been incorporated in clone trials.

A subset of the Berrimah hedges (the top 220 based on 6-month heights in clone trials planted in the NT in 2005), was also established as rooted cuttings clones in potted hedge gardens in Qld, initially at Gympie in 2006 then transferred to Mareeba; ultimately 153 hedges were planted in the field at Walkamin in 2009 (Table 4). If required, all or sub-sets of the hedges in the NT and Qld could be rejuvenated by basal coppicing.

Table 4. Inventory (2014) of *Khaya senegalensis* hedge gardens and clone and progeny trials established by month and year of planting in the Northern Territory (NT) and Queensland (Qld) at locations shown in Figure 2.

Hedge garden or Trial type (plant type)	Location	No. of Entries (excluding Control bulks)	Month, year of first planting	Plant owner, Land owner	Approx. net area (ha)
Hedge garden[a] (seedlings, ramets)	Berrimah, near Darwin, NT	630	Mar., 2004	Public, Public	NA[b]
	Walkamin, near Mareeba, Qld	153 (sub-set of above)	Dec., 2009	Public, Public	NA
Clone trial (rooted cuttings clones)	CPHRS[c], Darwin, NT in adjacent trial: A and B	A – 336 B – 188	Jan., 2005 Feb., 2005	Public, Public	3.0
	Katherine Research Station,NT	238	Jan., 2006	Public, Public	1.1
	Douglas Daly Research Farm (RF), N	63	Feb., 2006	Public, Public	0.4
	Douglas Daly, NT	197	Mar., 2007	Public, Private	1.4
	Near Ayr, Qld	153	Aug., 2007	Public, Private	1.6
	Abergowrie[d], near Ingham, Qld	56	May, 2008	Public, Private	1.0
	Douglas Daly, NT	320	Dec., 2008	Public, Private	1.7
	Near Mareeba, Qld	48	June, 2009	Public, Private	0.9
	Douglas Daly RF, N	141	Jan., 2010	Public, Public	0.7
	Near Mareeba, Qld	54	Jan., 2011	Public, Private	4.0
	Douglas Daly, NT	21	Jan., 2012	Public, Private	2.0
Progeny trial (seedling families)	CPHRS, Darwin, N (within clone trials)	A – 11 B – 9 (sub-set)	Jan., 2005 Feb., 2005	Public, Public	0.6
	Near Ingham, Qld	ca. 375 [e]	Oct., 2008	Private, Private	8.0
	Near Mareeba, Qld	12, 10 ex 10 CSO clones	June, 2009	Public, Private	0.6
	Near Katherine, NT	38, ex 30 CSO clones	Feb., 2010	Public, Public	2.6
	Near Ayr, Qld	42, ex 30 CSO clones	May, 2010	Public, Public	1.2
	Douglas Daly	14, ex 13 CSO	Dec., 2010	Public, Public	2.0

Research Farm, NT	clones			
Douglas-Daly, NT	42 [e], 14 ex 13 CSO clones	Jan., 2011	Public, Private	2.2
Douglas Daly, NT	17 [e]	Jan., 2011	Private, Private	0.6
Douglas Daly, NT	90 [e], 15 ex 15 CSO clones	Jan., 2012	Public and Private, Private	1.9
Near Ingham, Qld	44[e], 13 ex 13 CSO clones	May, 2012	Public, Private	0.6

[a] A hedge garden from bulked seed of local selected trees established at Weipa, Qld in 2004 was lost to land redevelopment

[b] Not available

[c] CPHRS – Coastal Plains Horticultural Research Station

[d] Trial lost due to land re-development in 2014 after severe damage from Tropical Cyclone Yasi in 2011

[e] These Entries are or include first-generation families from African seed

Clone Trials

Clones were developed as rooted cuttings from sub-sets of hedges 1 to 536, either in the NT or Qld, for establishment of clone trials planted in the NT (8) and Qld (4) during 2005–2012 on government land or collaboratively on private and company lands (Table 4). Within-clone replication in the NT trials increased over time as proliferating shoots on hedges yielded more cuttings, the number reaching eight ramets per clone for 50 clones of the 320-clone trial planted in the Douglas Daly in December 2008 (Table 4). Trials in Qld, being derived from several hedges per clone, had higher replication – commonly more than10. Among trials, numbers of clones vary from 21 to 336 (Table 4). Low numbers of clones and of replications for some clones partly reflected clonal variation in rootability (Reilly et al. 2011). Approximately 400 clones are under test across the NT and Qld trials, the latter including a clone of a Qld select tree. Almost all 44 parents of hedges are represented in clone trials.

Progeny Trials

Progeny trials were established on government, company or other private land between 2005 and 2012 (Table 4). They generally incorporate first- and advanced-generation families; for convenience families of all generations are documented together in Table 4. All the progeny trials are of open-pollinated families, either from seed of African or Australian ortets (original parents), or ramets (CSO grafts) of Australian selects. Families from open pollination of ortets (OPO) are from selected trees as follows: 11 Darwin, 3 Qld and 40 Kununurra trees (Table 5). These 54 families trace to at least five known provenances from Burkina Faso, Ghana, New Caledonia and Senegal and provenances of unknown origin.

CSO seedlots collected from the NT and Walkamin CSOs have been incorporated into seven progeny trials deployed across the NT and Qld during 2009-2012 (Table 4). This seed, referred to as 'open pollinated from ramets' (OPR), was kept separate by individual ramets. Across the trials, a total of 40 CSO clones are represented (Table 5). One parent tree is represented by both OPR and OPO families. There is scope for progeny testing of many more of the 162 untested CSO clones (see Table 1).

Across the OPO and OPR progeny trials a total of 93 unique families are deployed (Table 5). They trace to 16 known provenances across the species natural range representing nine African countries (Burkina Faso, Central African Republic, Ghana, Ivory Coast, Nigeria, Senegal, Sudan, Togo and Uganda) and three from New Caledonia and unknown (Table 5).

Summary of Advanced-Generation Genetic Resources

Progeny trials include families of a net 93 unique, selected Australian trees – 54 as OPO families and 40 as OPR families (with one select tree represented as both OPO and OPR families) (Table 5). Additional unique parents are represented in clone trials as: cloned seedling progeny of an estimated 30 parents of unknown pedigree (ca. 20 Weipa trees and ca. 10 Darwin trees); plus clones of three Darwin trees and a single Qld select tree, for an estimated total of 34 additional unique parents. Overall therefore, advanced genetic resources of Ks in Australia comprise seedling or cloned progeny from an estimated 127 unique parent trees from 16 known provenances of 9 African countries (listed above), unknown African provenances and three from New Caledonia (Table 5). There may be a level of co-ancestry among these genotypes, e.g. where more than one first-generation parent had been selected in a provenance. Tactics potentially available to limit co-ancestry in new breeding and deployment populations were mentioned above under ***Inventory of Recent Provenance Trials***. Secondary resources could be broadened by establishing trials from seed of the 162 orchard clones not yet represented in progeny trials, and seed of new selections.

Table 5. Numbers of parents of advanced-generation Australian families from open pollination of local ortets (OPO) or of ramets (OPR, within clonal seed orchards) by country and provenance represented in progeny trials

Country	Provenance[a]	Location of stand/s of parent tree selection	OPO families	OPR families
Burkina Faso	D416	Darwin, Northern Territory	1	1
Central African Republic	D391	As above		2
Ghana	D500	As above	2	1
Ivory Coast	S10050	As above		3
Nigeria	D480	As above		4
	D486	As above		3
Senegal	D417	As above	3	3
	S9392	As above		3
	S10066	As above		1
Sudan	S9368	As above		1
	S9687	As above		1
Togo	D411	As above		2
Uganda	D407	As above		3
	D408	As above		1
	S9620	As above		2
	S10053	As above		2
Unknown (Africa)	Unknown	Burdekin Agricultural College, near Ayr, Queensland	3	
	As above	Darwin, Northern Territory	1	2
	As above	Kununurra, Western Australia	40	
New Caledonia	D477	Darwin, Northern Territory		1
(ex Ivory Coast)	D487	As above	3	1
	D522	As above	1	3
Totals	19 + Unknown		54[b]	40[b]

[a] Alpha-numeric names are the seed accession labels given to the seedlots on arrival in Australia – D for those received at Darwin directly, S for those received via CSIRO's Tree Seed Centre, Canberra; details of geographic origins of the provenances in Africa and numbers of parent trees sampled not provided

[b] One parent has both OPO and OPR families. Hence the total number of unique parents is 93. An estimated 34 additional unique parents are represented as cloned progeny bringing the grand total to an estimated 127 unique Australian parents represented in advanced-generation progeny trials in Australia

Significance of the Genetic Resources of Ks in Australia

Include Range-Wide Provenance Collections Difficult to Replicate

The primary genetic resources of Ks established in Australia since the 1960s, mainly in the mid- to late-2000s, are from seed collected across the natural range of the species representing more than 142 provenances from 17 of the 20 countries in which it occurs naturally (Table 2). This large *ex situ* collection of germplasm is important because it has become difficult to secure range-wide provenance collections due to human conflicts in several African countries. Also, the genetic resources of Ks in Africa continue to be depleted through habitat loss and over exploitation reducing the gene pool available for new sampling.

Are Relatively Secure

While there is risk of further loss of genetic resources of Ks in northern Australia due to fire, cyclone or land redevelopment, it is mitigated by a number of factors. The 202 clones (Table 1) are distributed among five CSOs at disparate locations in the NT and Qld (Figure 2) giving a degree of security against loss of all clones. Many of these first-generation selections are also represented in clone and progeny trials on government, private or company land that is protected from fire within larger estates; this also applies to the progeny trials of more than 400 families from African seed (see Table 4). While the provenance trials are deployed more narrowly, the largest and several smaller ones remaining are located within the well-protected industrial plantations of the Douglas Daly, NT where cyclones are rare.

However, losses have occurred as described in earlier sections of this paper and there could be further losses. Hence there is a need to safeguard elements of the broad-based resources remaining. The challenge is to undertake wise management of the Ks germplasm established in Australia. We consider this is most likely to succeed via a collaborative, recurrent selection breeding program that provides improved material primarily for commercial deployment (Dickinson 2011). Incorporation of selected trees, from across the whole range of the genetic resources of Ks in Australia, as grafts in CSOs and as progeny in seedling seed orchards that are cycled over time, would most likely be components of such a breeding program that may be rekindled when investment conditions are favourable. Ideally, establishment of such seed orchards would pre-empt the next phase of commercialisation of Ks in Australia thus likely reducing the lag time to availability of improved seed.

Exhibit High Genetic Variation

Data analysis of clone trials to age 7.4 years show significant differences among clones for all traits measured (unpublished reports of J De Faveri, DAF, Mareeba). For example, among the 153 clones in a trial near Ayr, Qld, mean diameters at age 7.4 years ranged 7.7–18.1 cm, a 135% difference (Lindsay 2015a). Most clone trials included a bulk seedlot from Darwin street trees and an African provenance as Controls the latter performing better than the former for most traits. While 'all clones' means exceed the African Control for most traits, the means for the 'top 10%' of clones invariably surpass the African Control. Results of clone trials have enabled tentative identification of promising clones that could be considered for inclusion in new CSOs and breeding populations. However, outstanding clones are difficult to determine (Reilly et al. 2011).

Results of assessments of an advanced-generation progeny trial near Mareeba, Queensland (on a very poor site) at age 5.5 years indicate much variation among the 12 families for all traits assessed. Mean diameters of the families ranged 5.6– 7.4 cm, a 32% difference (unpublished data). In a trial near Ayr, Queensland (on a poor site) measured at age 3.3 years, mean diameters of the 41 CSO families ranged from 5.7– 8.1 cm, a 42% difference (unpublished data). In both trials, the 'all families' means surpassed the African Control for most traits while the 'top 10%' of families did so invariably. Means for DBHOB and height of the 10 families with seed parents originally of western-region provenances (Burkina Faso, Ghana, Ivory Coast and Senegal) were similar to those of the 15 families from far-eastern original sources (Sudan and Uganda), but the western-provenances families had better bole length, straightness and Value index (defined as $DBHOB^2$ x bole length x straightness score). These and other progeny trials have enabled tentative identification of diverse superior families (and hence the better CSO clones to target for re-collection of seed) and of promising individual advanced-generation trees. Promising parents and superior individual trees in both OPO and OPR progeny trials could be considered for inclusion in next-cycle seed orchards and breeding populations.

High provenance variation is demonstrated by results of two large trials established in the NT and Queensland in 2008 (38 provenances from Burkina Faso, Cameroon, Guinea, Mali and Senegal plus Australian Controls), and of another trial planted in the NT in 2009 (71 provenances from Benin, Burkina Faso, Cameroon, Mali, Niger and Senegal). These provenances span a wide range of environments in Africa: elevations (9m–543m above sea level), mean annual rainfalls (500mm– 1,300mm) and geographic distributions (10°N in Cameroon to 14.75°N in Mali and 16.5°W in Senegal to 16.5°E in Cameroon). All the several economic traits assessed[8] at ages 3 years and 4.3 years respectively exhibited statistically significant and economically important differences among provenances (Dickinson et al. in prep., M. and S. Carson 2014, Fremlin 2014). For example, provenance means for diameter in the 2008 Queensland trial ranged from 6.9 cm for a Mali provenance to 9.3 cm for a Burkina provenance, a 35% difference. While particular Australian and African provenances were equally good for height, diameter and disease incidence, best Australian provenances were inferior to the best African provenances in straightness, branch size, axis persistence and wind firmness. In the 2009 NT trial the range in diameter was from 6.0 cm for a Niger provenance to 8.6 cm for a Burkina Faso provenance, a 43% difference. Photographs in Fremlin (2012) illustrated great differences in growth and tree form between provenances grown in the NT.

Confirming Australian results, significant provenance variation was exhibited for tree volume, axis persistence and stem form at 3.5 years of age in a trial planted in Sri Lanka in 2008 with a 21-provenance sub-set (Senegal to Niger) of the 71 provenances in the 2009, NT trial, with far-western provenances the more promising (Bandara 2014, Kangane 2014).

Include Large Numbers of Promising Provenances and Individual Trees for Screening
Most of the promising provenances for growth and tree quality traits in both the Australian and Sri Lankan provenance trials mentioned above were from the western region of the Ks range in Africa. One hundred provenances from across this western region are established in Australian plantings representing seven countries - Burkina Faso, Gambia, Ghana, Guinea, Ivory Coast, Mali and Senegal (Table 2). However, individual trees combining good growth and stem quality have been observed in all provenances (Fremlin 2014).

Furthermore, at older ages when additional biotic and abiotic factors may have impacted the populations available for domestication, indications of relative performances of provenances may change. Therefore, it would be wise to select trees for future conservation and breeding populations from a wide range of provenances to assure high diversity in these populations.

Large numbers of individuals from high performing populations with high diversity are needed for sustainable domestication (White et al. 2007). Within most of the provenance trials, substantial numbers of trees are available for screening to select superior trees for conservation and tree improvement. Our experience shows that large numbers of trees have

[8] Traits assessed in one or more of the Australian trials included diameter, height, stem straightness, branch thickness, axis persistence, wind firmness, diseased trees ('pink' disease) and 'tree value index'

to be screened to identify superior trees meeting the criteria of the tree improvement objectives for Ks defined by Dickinson et al. (2009). In relation to veneer production, they stated (p 5): 'The straighter, the larger in diameter and the lower the incidence of knots in logs, the greater the recovery of high-value veneers'. (These criteria should also include 'the lower the log taper'). It is fortunate there are very large numbers of trees across the provenance and other trials and commercial plantings of Ks in Australia, allowing high selection intensity.

While the Australian provenance, clone and progeny trials together cover more than 100 ha of plantations (Tables 3, 4), and phenotypic selection is expected to be relatively accurate within the clone and progeny trials (half-pedigrees mostly known), there are far greater numbers of trees across the industrial plantations and woodlots which would enable very intensive selection. Although pedigrees of trees selected in these plantings would be unknown, decisions on how to use potentially-related trees effectively might be made as mentioned above under ***Inventory of Recent Provenance Trials***. Such knowledge would also provide a partial basis for restricting co-ancestry when considering thinning existing clone and progeny trials to develop seed orchards.

Allow Identification of Promising Parents, Clones, Families and Individual Trees in Trials

First-generation progeny trials of more than 400 African trees and advanced-generation clone or progeny trials of an estimated 127 unique local parent trees are established in northern Australia (Tables 4, 5). All of the economic traits assessed in several advanced-generation trials at ages 7.5 years and 5.5 years exhibit statistically significant and important differences among clones and families (unpublished reports of J. de Faveri, DAF, Mareeba). This has enabled identification of promising clones, families and individual trees at specific trial sites. However, the impact of genotype-by-environment interaction for these promising materials is currently unknown. These progeny and clone trials allow greater accuracy of individual tree selection for the next cycle of breeding and deployment than is possible in the provenance trials and industrial plantations where pedigree is not known. Such promising material warrants inclusion in future breeding and seed producing populations that are large enough to allow culling of, for example, genotypes exhibiting unfavourable environmental interaction.

Provide a Good Base for Genetic Improvement and Plantation Expansion

"Genetic diversity is widely recognised as the key component for long-term survival of most tree species" (Gapare 2014, p 312). In domesticating a species the strategic importance of a broad base of genetic resources including high-performing provenances is well known, e.g. White et al. (2007), Dvorak (2012). There is great variation among the diverse clones, families and provenances of Ks in respective Australian trials (see the section **Exhibit High Genetic Variation** above). Such high genetic variation, and the high phenotypic variation observed between individual trees (e.g. Lindsay 2015b, Slide 16), bodes well for further domestication, including via establishing 'seed production areas' ; collecting seed from superior phenotypes, proven CSO clones and 'best clones' in trials; and establishing new seed orchards. [One replication of the provenance trial planted in the Douglas Daly in January 2008 (see Table 3) has been converted to a 'provenance seedling seed orchard' (*sensu* Nanson 1972) that is being managed by thinning and irrigation (Fremlin 2015)].

However, repeated cycles of selection narrow the genetic base and may eliminate some genes that are needed later (Libby 1973). This problem may be addressed by retaining a fairly broad range of genotypes in breeding populations and a more select group in deployment seed orchards (White et al. 2007). Local availability of a broad genetic base of a species under domestication enables responsiveness to changes in selection goals in tree improvement when, for example, pest and disease issues arise, or abiotic changes occur, or new products are sought by consumers. The large, diverse and high-performing genetic resources of Ks in Australia should enable future challenges to be met successfully.

Constitute a Potential Source of Germplasm for Other Programs and Possible Re-introduction

As well as serving local domestication efforts, the genetic resources of Ks in Australia could potentially contribute germplasm for infusion into existing or founding new programs elsewhere. For parts of the natural range in Africa where genetic resources of Ks have been very severely eroded, Australian material of known provenance could be considered for re-introduction and refreshment of those depleted resources, conditional on appreciation that most open-pollinated Australian seed could represent inter-provenance crosses. Re-introduction of genetic material of some *Pinus* species to Central America and Mexico has been undertaken by CAMCORE (Dvorak 2012) and similar action is under consideration for return of some provenances of *Eucalyptus urophylla* to Indonesia (Dvorak et al. 2008).

Conclusions

Large areas and numbers of African provenances and families of Ks have been established in northern Australian plantations, and numerous advanced-generation clones and families have been derived from the early introductions. This resource is likely to comprise the broadest genetic base of Ks in a single country outside Africa. It includes large numbers of provenances from the western region of the range of the species in Africa which are generally the more promising at sites in northern Australia. The great genetic variability for economic traits within these resources, shown by the results of trials mentioned in the present paper, and their high molecular diversity, together with the vast numbers of Ks trees present in northern Australia, should enable local selective breeding and conservation to proceed successfully.

Since some loss of genetic resources of Ks have occurred in Australia, there is a need to safeguard elements of the broad-based resources remaining through wise management. Based on other experience we suggest that a collaborative, recurrent selection breeding program that provides improved material primarily for commercial deployment should be considered for implementation by stakeholders as soon as possible.

The genetic resources described could also serve as a large *ex situ* reservoir of the germplasm of the species for infusion into existing programs elsewhere or initiation of new programs, or even conditional re-introduction to parts of Africa. In the Australian context, the wide range of primary and advanced-generation germplasm established in northern Australia and documented in this paper, provides a sound basis for further domestication and industrial plantation and woodlot expansion, when investment conditions are favourable.

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